Vol 4/2 (2004) 174 - 178

JOURNAL OF NATURAL REMEDIES

# Non-Opioid anti-nociceptive effect of *Psidium guajava* leaves extract

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**Abstract**

Objective: The main aim was to demonstrate the anti-nociceptive effects of *Psidium guajava* leaves extract. Methods: Ethanol extract of *P. guajava* leaves was administered intraperitoneally to mice 30 min before administration of acetic acid. The number of abdominal constriction was noted. Results: *P. guajava* leaves extract showed statistically significant decrease in abdominal constriction (writhing) when compared to controls. The percentage inhibition was 20.9, 53.9 and 97.3 for 2, 3 and 4 mg/kg extract respectively. These mice showed normal behavior and motor activity. The antinociceptive effect was statistically similar to equivalent doses of mefenamic acid. Morphine, an opioid analgesic revealed 29.0, 57.3 and 100.0 inhibition of abdominal constriction for 0.2, 0.3 and 0.4 mg/kg respectively. Interestingly, pretreatment with Naloxone did not significantly decrease the analgesic effect of *P. guajava* leaves extract. Conclusions: Results from this present study revealed *P. guajava* extract exerted a potent anti-nociceptive effect, which was similar potency to mefenamic acid and 10 times less potent to morphine. The antinociceptive effects were dose-dependent, without behavior changes and may not involve the opioid receptors.

**Key words:** *Psidium guajava*, Antinociceptive, Morphine, Naloxone, Mefenamic acid

## 1. Introduction

*Psidium guajava* Linn. also known as the guava plant or "Jambu batu" in Malaysia has been used extensively in local traditional medicine. *P. guajava* is native to most of the equatorial and tropical type climate countries. [1] It has been reviewed that *P. guajava* leaves able to relief fever, dysentery and pain [2] has antimicrobial activities [3], treating ulcers, wounds and many digestive tract dysfunctions [4]. The flowers are used as antipyretic, treatment of bronchitis and eye-sore [5]. The fruit has been reported to possess hypoglycemic activity, [6] and a good source of vitamin C [7]. Interestingly, in Indonesia, leaves extract is used to treat swelling and controlling pain during childbirth [1]. In China, the leaves are used as anti-inflammatory agent [8].

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Leaves from *P. guajava* contained more than twenty identified compound includes sesquiterpenes hydrocarbons, the major sesquiterpene is caryophyllene (Figure 1) and others such as myrecene, limonene, langi-cyclene, eugenol, benzaldehyde, nerolidol [1].

Other compounds isolated include flavonoids, tannins and saponins [3]. However, a different group of researchers reported no alkaloids and antraquinones were detected in *P. guajava* leaves [9].

As the leaves are used traditionally for controlling pain during birth and dysentery, the objective of this present study was to evaluate the effects of *P. guajava* to inhibit pain (antinociceptive effect) in acetic acid induced abdominal writhing in mice.

## 2. Materials and methods

### *2.1 Drugs*

Morphine sulfate and naloxone hydrochloride were gifts from Department of Pharmacology, Faculty of Allied Sciences, National University of Malaysia. Mefenamic acid, glacial acetic acid and all other chemicals were purchased from Sigma Chemicals, Dorset, England.

### *2.2. Plants and Extraction*

The leaves of *Psidium guajava* were collected from Sepang District, Selangor, Malaysia. The plants/leaves were verified by Associate Professor Dr. Mohd Said Saad of Plant Genetics Unit, Institute of Bioscience, Universiti Putra Malaysia. Voucher specimen was deposited at the Phytomedicinal Herbarium, Institute of Bioscience, Universiti Putra Malaysia. The leaves were then oven dried at 45°C overnight, grounded into powder form using the grinder and extracted using a Soxhlet apparatus with 95% (v/v) ethanol as solvent for 12 h as previously described [10] (Somchit *et al* 2003). The resultant extraction was dried using a rotor-evaporator for 4 to 6 h.

### *2.3 Animals and Treatment*

100 male Bulb/C mice (30-40g body weight) were used in this study. They were housed in plastic cages in groups of ten with wood shaving as bedding. Feed and water were available *ad libitum*. All procedures in this study were approved by the University Ethical Committee.

The mice were divided into control group (given 0.25 mL normal saline ip), *P. guajava* extract groups (2, 3 and 4 mg/kg at 0.5 mL/mouse ip) and positive control group (morphine at 0.2, 0.3 and 0.4 mg/kg at 0.5 mL/mouse ip or mefenamic acid at 2, 3 and 4 mg/kg ip) 20 min prior of giving 0.5 mL 0.6% (v/v) acetic acid ip. Abdominal contractions were recorded after half an hour for 15 min. Another group of mice was given Naloxone (a non-specific opioid antagonist) at 10 mg/kg prior to 3 mg/kg *P. guajava* extract administration.

### *2.4 Statistical Analysis*

Results were expressed as mean ± sd. Statistical significance was determined using analysis of variance (ANOVA). Values of $p<0.05$ were considered significant. For significant treatment means obtained by ANOVA, they were subjected to Duncan multiple post-test.

## 3. Results

Various concentrations of *P. guajava* extract were examined in order to establish a dose-response profile. Intraperitoneal injection of *P. guajava* extract showed statistically significant decrease in abdominal constriction (writhing) when compared to controls. There was demonstrable dose-response pattern of the activity of the extract at 2 to 4 mg/kg. The percentage inhibition was 20.9, 53.9 and 97.3 for 2, 3 and 4 mg/kg extract respectively (Table 1). There was statistical significance

Table 1
Effect of *Psidium guajava* extract on abdorminal constriction response

| Treatment Group | n | Mean abdorminal constriction | Percentage inhibition |
|---|---|---|---|
| Control | 10 | 99.5 ± 20.1[a] | 0 |
| 2 mg/kg *P. guajava* | 10 | 78.7 ± 8.0[a] | 20.9 |
| 3 mg/kg *P. guajava* | 10 | 45.8 ± 10.2[b] | 53.9 |
| 4 mg/kg *P. guajava* | 10 | 3.7 ± 2.0[c] | 97.3 |

[a-c] Means within a column no common superscripts differ significantly ($p<0.05$)

($p<0.05$) even when compared between the three treatment means. There were no behavioral changes in the mice during the study period for all three doses of *P. guajava* extract treatment.

Table 2 demonstrates the antinociception of morphine, which is a potent opioid analgesic. It inhibited acetic acid induced writhing as low as 0.2 mg/kg in concentration and total inhibition (zero writhing) were observed at the dose of 0.4 mg/kg. The mice given morphine exhibited sluggish motor activity, depression, produced sedation and sleep. Animals pretreated with mefenamic acid revealed a dose-dependent antinociceptive activity. These animals also had no behavioral changes.

Table 2
Opioid and Non-opioid antinociception in mice

| Treatment group | n | Mean abdorminal constriction | Percentage inhibition |
|---|---|---|---|
| Control | 10 | 99.3 ± 17.2[a] | 0 |
| 0.2 mg/kg Morphine | 10 | 70.65 ± 10.3[a] | 29.0 |
| 0.3 mg/kg Morphine | 10 | 42.48 ± 15.1[b] | 57.3 |
| 0.4 mg/kg Morphine | 10 | 0 | 100 |
| 2 mg/kg M. Acid | 10 | 63.7 ± 13.1[a] | 36.4 |
| 3 mg/kg M. Acid | 10 | 37.3 ± 9.8[b] | 62.6 |
| 4 mg/kg M. Acid | 10 | 4.5 ± 1.7[c] | 95.9 |

M. Acid=mefenamic acid
[a-c] Means within a column no common superscripts differ significantly ($p<0.05$)

Interestingly, pretreatment with naloxone did not significantly decrease the analgesic effect of *P. guajava* extract. The group of mice given 3 mg/kg *P. guajava* extract and mice given 10 mg/kg naloxone + 3 mg/kg *P. guajava* extract had statistically similar ($p>0.05$) number of abdominal constrictions (Table 3).

## 4. Discussion

Results from this present study indicated that *P. guajava* leaves contain antinociceptive property that was similar to the effect of mefenamic acid, a nonsteroidal anti-inflammatory drug. This drug is used for its anti-pyretic, anti-inflammatory and analgesic properties. The antinociceptive effect was observed without any changes in the behavior of the mice. This antinociceptive response was observed to be dose - dependent.

Experiments with the opioid receptor antagonist, naloxone revealed that this antagonist had no effect on the antinociceptive effect of the leave extract. Therefore, it can be suggested that the antinociceptive effect of *P. guajava* leaves extract may not involve the opioid receptors and may involve some other non-opioid mechanism. However, the exact mechanism is still unknown.

Table 3
Effects of Naloxone (Opioid receptor antagonist) on the antinociceptive effect of *Psidium guajava* extract

| Treatment group | n | Mean abdorminal constriction | Percentage inhibition |
|---|---|---|---|
| Control[1] | 10 | 99.5 ± 20.1[a] | 0 |
| 3 mg/kg *P. guajava* | 10 | 49.3 ± 13.7[b] | 50.5 |
| 10 mg/kg Naloxone + 3 mg/kg *P. guajava* | 10 | 37.5 ± 7.9[b] | 62.3 |

[1] Data from the control group from Table 1.
[a-b] Means within a column no common superscripts differ significantly ($p \leq 0.05$)

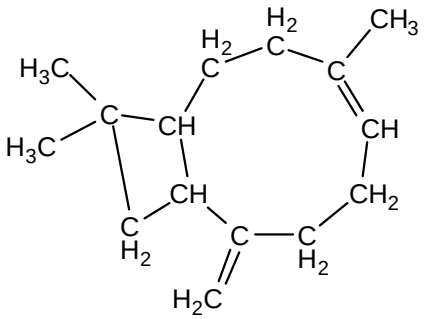


Fig. 1. Caryophyllene, major sesquiterpene from *Psidium guajava*

Olajide *et al*. [11] reported pharmacological studies of *P. guajava* leaves. The methanol extract of *P. guajava* leaves contained anti-inflammatory, analgesic and anti-pyretic activities. The findings from this current studies using ethanol extraction were in agreement with this previous study, where analgesic/ antinociceptive effects were observed.

However, they used higher doses of 50 to 200 mg/kg. Therefore, the phyto-compound/s that is/are responsible for antinociception are extracted via ethanol extraction.

The antinociceptive effects were probably due to essential oil present in the guava plant [12] and could also be due to the high flavonoid content [9]. A closely related species, *Psidium guianense* essential oil has been reported to possess analgesic and anti-inflammatory effects [13]. The guava leaves contained caryophyllene, a sesquiterpenes (Figure 1).

This compound has been proposed to posses analgesic activity. Indeed, Ghelardini et al (2001) [14] reported local anesthetic activity of caryophyllene isolated from flowerbuds of Syzygium aromaticum in rabbits. The antinociceptive activity of *P. guajava* leaves was approximately 10 times less potent than morphine.

Although less potent, the *P. guajava* extracts were void of behavioral changes such as lack in motor action and sedation, which was evident in mice given morphine. Future studies must be performed to determine if any dependence or withdrawal syndrome associated with *P. guajava* extract. However, this may not occur due to *P. guajava* acts on a pathway free of opioid receptors.

*P. guajava* has been mainly used for treatment of many types of gastrointestinal ailments from infection to dysentery [15]. However, the data presented in this current study suggested that *P. guajava* has antinociceptive property, which might be potentially useful herbal analgesic agent without any behavioral changes or loss of consciousness.

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